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TERTIAN MALARIAL FEVER.

TRANSMISSION EXPERIMENTS WITH ANOPHELES PUNCTIPENNIS.

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The first experimental proof of the infection of *Anopheles punctipennis* Say by *Plasmodium vivax* is offered by King in a recent number of the American Journal of Tropical Diseases and Preventive Medicine.¹ Two experiments are described in which seven mosquitoes were induced to feed, resulting in infection of six, four with oocysts on the stomach wall and two with gland and stomach sporozoites; infection developed after a period of 7 to 31 days.

The present account of work carried on in connection with malarial investigations under the direction of Surg. R. H. von Ezdorf describes experiments confirming King's findings and further extending the investigation with a greater number of specimens, in addition to presenting positive results of human infectibility experiments with the same species. Although it has been commonly accepted that the presence of gland sporozoites in a given species of *Anopheles* is sufficient to incriminate that species as a potential vector of malaria, human control experiments were considered desirable. The wide distribution of *Anopheles punctipennis* in the United States and its demonstrated relations to matters of public health will require its inclusion among mosquitoes against which antimalarial measures should be directed in the future.

Laboratory reared mosquitoes were unfortunately not available at this time; it is realized that these are essential in order to eliminate the possibility of previous contamination. Less suitable material was employed in these experiments, as "wild" *Anopheles* collected during the early part of January were used. The improbability of infection of "wild" *Anopheles* mosquitoes in this section and during this season seemed to warrant their use in conducting these experiments.

For the purpose of this study, 388 specimens of *Anopheles punctipennis* were collected in barns and stables at Talladega Springs, Ala., January 7-15, 1916, and transported in cages to the New Orleans laboratory. The feeble and dead mosquitoes were withdrawn upon arrival and either used for smear preparations or dissected in the usual manner for the purpose of examination for malarial parasites. One hundred and sixty-six mosquitoes were separately suspended in a drop of saline dissecting medium on a slide and the abdominal and thoracic contents were teased out. Smears were then made from each and stained over night in a weak Giemsa stain.

¹W. V. King (1915), *Anopheles punctipennis*, A Host of Tertian Malaria. American Journal of Tropical Diseases and Preventive Medicine, vol. 3, No. 8, February, 1915, pp. 426-432.

One hundred and twenty-six specimens were fed on a healthy person at least once each, then dissected during a period of 20 days. The results in all instances failed to indicate the presence of any plasmodial infection.

An untreated patient suffering from tertian malarial fever volunteered for the purpose of furnishing the parasites required in this experiment. During the interval of awaiting gametocyte development (Jan. 17-Feb. 6) several generations of complete schizogony were observed to occur in the patient's blood. However, sporogony was unduly inhibited up to January 29, too few gametocytes being present to assure infectivity of the definitive hosts. Finally, sufficient numbers of sexual forms matured during January 29 to February 6, at the termination of which period (Feb. 6 and 7) mosquitoes were applied to the patient.

The paucity of sexual parasites in the blood of the donor may be appreciated from the counts made in a thick film and a thin film prepared February 7. In the two preparations 1,231 leucocytes were counted and the matured gametocytes encountered numbered two, an average of one gametocyte to 616 leucocytes. In addition to these there were observed, in the two blood specimens, 48 half-grown gametocytes and 5 ranging in size from three-fourths to nearly full-grown forms. At the time of these examinations the patient had been started on a course of quinine treatment.

Forty specimens of *Anopheles punctipennis* were applied to the tertian donor in two lots on February 6 and 7, 1916; 20 of these died within five days and were dissected. Ookinetes were observed in at least eight of these, from which smear preparations were made. Twenty mosquitoes were dissected during a developmental period ranging from 6 to 25 days.

During the course of the experiment raisins and water were furnished as food, while the mosquitoes were subjected, prior to the 10th day, to an incubator temperature of 25.5°-26° C. Thirteen of this lot of 40 (equal to 32.5 per cent) became infected. The following protocol describes the degree of parasitism in each of the mosquitoes concerned:

No. 1. Dissected February 13, 1916. Incubation, six days. There were present four probably one-third grown oocysts (20 microns or less) apparently of the same age. One only showed a small amount of malarial pigment, the remainder lacking pigment, but with undifferentiated protoplasm, presporoblastic in character. The four oocysts were situated near the posterior end of the mid-gut. The six lobes of the salivary glands had a normal appearance.

No. 2. Date of dissection, February 14. Seven days' incubation. There were eight fairly well-developed oocysts situated between the middle and posterior end of the mid-gut. None of the bodies possessed pigment. A few (3) appeared with indistinct sporoblasts, the remainder being undeveloped with slightly mottled or reticulated contents. Five lobes of the salivary glands were examined; they appeared devoid of parasites.

No. 3. Date of dissection, February 14. Seven days' incubation. Eleven oocysts were seen on the mid-gut, the majority situated near the posterior end; four contained sporoblasts, though not well defined. Seven were presporoblastic in structure, though lacking malarial pigment. The salivary glands gave no evidences of parasitism.

No. 4. Dissected February 15. Incubation period, eight days. Five oocysts were seen, all found on the posterior end of the mid-gut. One contained a very small amount of pigment. This was the smallest parasite present. The others showed thin-walled capsules without sporoblasts. The salivary glands were negative.

No. 5. Dissected February 16. Nine days' incubation. On examination this mosquito showed 22 to 24 three-quarter grown oocysts, the majority appearing with definite sporoblasts; none showed a spiculed structure or sporozoites. The salivary glands, likewise, were not parasitized.

No. 6. Dissected February 16. Incubation 10 days. This contained six perfectly developed oocysts, with ripe sporozoites. One of the capsules ruptured under observation, resulting in a swarming of motile filaments in the saline suspension. In addition, two oocysts were present with only sporoblasts. Only three lobes of the salivary glands were dissected out. Two of these were jammed and matted with innumerable full-grown sporozoites, many of which were observed to escape through the gland cells into the saline dissecting medium.

No. 9. Dissected February 17. Incubation of 10 days. Only one shrunken oocyst with its contents expelled was found on the stomach wall. Both salivary glands were examined; the six lobes were heavily parasitized and a great number of free sporozoites were found in the mounting fluid.

No. 12. Dissected February 18. Incubation of 11 days. The stomach wall of this mosquito harbored two oocysts, one of which was much shriveled and both were empty of protoplasmic contents.

The salivary glands were moderately invaded with sporozoites which became active when set free in the mounting fluid.

No. 17. Dissected February 19. Twelve days' of incubation. Here no evidence of an oocyst was given, although the preparation was sufficiently clear to view the gut structures minutely. The salivary glands were invaded with a small number of typical sporozoites.

No. 18. Dissected February 19. Incubation 12 days. The structures of the midgut were fairly obscured by the projecting mass of oocysts. Twenty-eight of these bodies were counted; seven were observed to contain sporoblasts, while the majority of the remainder contained varying numbers of spindle organisms in different stages of development. Several of the bodies ruptured upon slight pressure, when swarms of relatively small sporozoites floated away from the ripe oocysts. The salivary glands in this specimen were heavily parasitized. Five of the lobes appeared granular with matted clusters of typical curved organisms. Numerous sporozoites were seen moving sluggishly through the saline medium.

No. 23. Dissected March 3. Incubation period 25 days. This specimen had been given a feeding of human blood five hours previously, consequently the alimentary tract was found engorged with blood. No oocysts or shrunken capsules were seen. Several sporozoites were seen moving freely in the solution surrounding the dissected stomach. The salivary glands were greatly hypertrophied; these lobes which in the dissection were torn loose with the head were parasitized with a moderate number of sporozoites, and a remaining single lobe afterwards removed from the thoracic cavity was seen to be greatly shrunken in size, its degenerated cells containing only a few sporozoites. A large number of these organisms were observed free and very active in some material dissected from the thorax.

No. 24. Dissected March 1. Incubation period 24 days. The midgut was seen with four oocystic capsules, three much shrunken, narrowed to probably one-half the width. One appeared normal in size, only slightly shrunken, containing three non-

motile sporozoit-like bodies. The dissected salivary glands were not removed cleanly from either the head or thorax. A small portion was attached to the head and two lobes of the glands were seen in material crushed from the thorax. On pressure, numerous very active sporozoits were observed. The gland structure was granular but no organisms were perceived in the cells on account of the presence of the mat of overlying fungous threads.

No. 25. Dissected February 23. Incubation period 16 days. The stomach of this specimen was not seen on account of the superimposed tangle of mycelium threads of some fungous parasite which probably caused the insect's death. When the gut wall was pressed a few sluggishly motile sporozoits were observed. These originated, probably, from a gut oocyst. Only small disjointed portions of the salivary glands were examined. These appeared heavily parasitized with more or less active sporozoits possessing the typical large refractile nucleus and sharply curved ends.

Nineteen specimens of *Anopheles crucians* Wied. were fed at the same time and kept under identical conditions with the *punctipennis*. Seven of these died within five days after feeding; three of these were found to contain numerous immature zygotes, and in one that was found dead on the second day, the ookinete stage was identified in the crushed stomach contents.

Two of the 12 surviving specimens of *Anopheles crucians* were found to be parasitized 11 and 13 days, respectively, after biting the blood donor. Both mosquitoes had a few shrunken oocysts and in only one of these were sporozoits observed. The salivary glands of one appeared granular with numerous clusters of filiform organisms distributed throughout the gland cells. In the other mosquito, only the terminal cells appeared invaded by a moderate number of sporozoits. In both specimens the sporozoits were typical in appearance, showing various degrees of activity, when the cover glass was pressed upon the parasitized glands.

Two specimens of *Anopheles quadrimaculatus* Say used as additional controls under the same conditions proved to be negative.

In order to eliminate any possibility of doubt as to the nature of the parasites harbored by the mosquitoes and as a further check on the infectivity of *Anopheles punctipennis*, three healthy persons volunteered to permit biting of these mosquitoes. Four specimens were selected for the purpose, namely, Nos. 18, 23, 24, and 25. The first volunteer (H. E. H.) was bitten February 17 by all four of the mosquitoes applied. At this time the mosquitoes had been infected 10 days. In this feeding, the mosquitoes were not permitted to engorge themselves but were applied a sufficient length of time to convince the observer and the host that blood was being withdrawn. Directly after the biting, six very distinct moderate-sized macules developed on the arm at the site of application. Nine days elapsed before the volunteer experienced prodromal symptoms of any kind. The first paroxysm was observed 14 days after the biting and parasites of *Plasmodium vivax* were found in blood of H. E. H. on March 3 and March 4.

H. E. H. was employed previously in feeding mosquitoes which had been given the opportunity of becoming infected in two experiments from subtertian crescent carriers. More than 200 specimens of *Anopheles punctipennis* had been used in an attempt to transmit subtertian infection, with negative results. The volunteer, H. E. H., remained healthy throughout this test and subsequently for four months prior to the tertian transmission experiment. In the tertian infection the disease took its usual course and prompt recovery followed the use of therapeutic doses of quinine.

Two of the mosquitoes, namely, Nos. 23 and 24, were induced to bite the second volunteer, Dr. H. A. T., February 18. These were observed to bite vigorously to a point of repletion. The mosquitoes at this time had been infected for a period of 11 days. After an incubation period of 14 days, Dr. H. A. T. experienced a distinct paroxysm; also on the same day parasites of tertian malaria (*Plasmodium vivax*) were found in his blood.

A third volunteer, Dr. R. C. D., was bitten February 21 by mosquitoes, Nos. 24 and 25, 14 days after they had received an opportunity to become infected. The two specimens were applied to the arm so that they did not become engorged (interrupted feeding); the labia of both were observed to be inserted to the extreme before the mouths were withdrawn. Each bite required 40 seconds of time, resulting in distinct macules at the point of inoculation.

An incubation period of 14 days followed in this case, with prodromata and the usual symptoms of chill followed by fever. A slight paroxysm was experienced on March 4, and parasites were observed March 5. Distinct tertian-rings (*P. vivax*) were demonstrated in the blood of the volunteer in two specimens examined at five-hour intervals on the second day after onset.

Summary.

1. *Anopheles punctipennis* has been proved to be a definitive host of tertian plasmodial infection. Under the conditions cited, 32.5 per cent of the 40 mosquitoes applied were positive as against 10.5 per cent of the *Anopheles crucians*. Only 20 specimens of *A. punctipennis* survived six days or longer after biting.

2. With a blood donor of low infectivity 13 mosquitoes of *A. punctipennis* showed infection from 6 to 25 days after securing parasitized blood. Eight of the positive specimens became infective as shown by moderate to immense numbers of typical sporozoits invading the salivary glands.

3. In a control series of 19 specimens of *Anopheles crucians*, two were infective with the parasites of *Plasmodium vivax* 11 and 13 days after biting the blood donor.

4. The infectibility of *Anopheles punctipennis* was demonstrated in the transmission of the disease through the biting of four mosquitoes which reproduced the infection in three healthy volunteers, living in New Orleans during February, 1916, after incubation periods of 14 and 15 days.

One of these persons had escaped infection four months previously from the bites of more than 200 *Anopheles punctipennis* in two experiments with the subtertian type. From this single instance it is indicated that *Anopheles punctipennis* served as a host of *P. vivax* but not of *P. falciparum*.

PLAGUE-PREVENTION WORK.

CALIFORNIA.

The following report of plague-prevention work in California for the week ended April 15, 1916, was received from Surg. Beggess, of the United States Public Health Service, in charge of the work:

SAN FRANCISCO, CAL.

RAT PROOFING.

New buildings:

Inspections of work under construction.....	206
Basements concreted (square feet 34,920)....	39
Floors concreted (square feet 19,625).....	16
Yards, passageways, etc., (square feet) 13,258	72
Total area of concrete laid (square feet)...	67,803

Class A, B, and C (fireproof buildings):

Inspections made.....	142
Roof and basement ventilators, etc., screened	325
Wire screening used (square feet).....	2,690
Openings around pipes, etc., closed with cement.....	350
Sidewalk lens lights replaced.....	700

Old buildings:

Inspections made.....	424
Wooden floors removed.....	46
Yards and passageways, planking removed.	12
New foundation walls installed (cubic feet) 5,857	
Concrete floors installed (square feet 38,015).	24
Basements concreted (square feet 29,430)....	33
Yards and passageways, etc., concreted (square feet 28,858).....	108
Total area concrete laid (square feet).....	96,303
Floors rat proofed with wire cloth (square feet 3,600).....	3
Buildings razed.....	25
New garbage cans stamped approved.....	605
Nuisances abated.....	278

OPERATIONS ON THE WATER FRONT.

Vessels inspected for rat guards.....	16
Reinspections made on vessels.....	18
New rat guards procured.....	2
Defective rat guards repaired.....	4
Rats trapped on wharves and water front...	23
Rats trapped on vessels.....	54
Traps set on wharves and water front.....	114
Traps set on vessels.....	49
Vessels trapped on.....	14

SAN FRANCISCO, CAL.—Continued.

OPERATIONS ON THE WATER FRONT—continued.

Poisons placed on water front (pieces).....	3,600
Poisons placed within Panama-Pacific International Exposition grounds (pieces).....	38,500
Bait used on water front and vessels, bacon (pounds).....	6
Bread used in poisoning water front (loaves)	9
Poison used on water front (pounds).....	3

RATS COLLECTED AND EXAMINED FOR PLAGUE.

San Francisco:

Collected.....	419
Examined.....	334
Found infected.....	None.

Monterey County:

Collected.....	4
Examined.....	4
Found infected.....	None.

RATS IDENTIFIED.

<i>Mus norvegicus</i>	201
<i>Mus rattus</i>	86
<i>Mus alexandrinus</i>	81
<i>Mus musculus</i>	51

SQUIRRELS COLLECTED AND EXAMINED FOR PLAGUE.

County.	Collected.	Examined.	Found infected.
Alameda.....	54	54	None.
Contra Costa.....	634	634	None.
Monterey.....	581	581	4
San Benito.....	186	186	1
Santa Clara.....	28	28	1
Total.....	1,483	1,483	6

Ten rabbits examined from Monterey County. Not infected.